

IN THE SPECIFICATION

Please amend the paragraph starting at page 6, line 32, and continuing through page 7, line 6, as follows:

In a particular and preferred implementation of the invention, the DNA chip according to the invention has a plurality (a number greater than or equal to 2, preferably 3, more preferably 5, most preferably 10) of oligonucleotides greater than 40 bases long. Preferably, said oligonucleotides comprise a fragment of at least 20, preferably 40 or 50, more preferably 75, most preferably 100 consecutive bases, of the sequences SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138 and SEQ ID [[No.]] NO: 140 to SEQ ID [[No.]] NO: 189, corresponding to the intergenic sequences of various species (rpoBC for SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138, GroESL for SEQ ID [[No.]] NO: 140 to SEQ ID [[No.]] NO: 189).

Please amend the paragraph beginning at page 7, line 24, as follows:

The method according to the invention is thus carried out using degenerate primers located in the coding sequences of the operons, in particular at least one primer chosen from the sequences SEQ ID [[No.]] NO: 1 to SEQ ID [[No.]] NO: 31, themselves a subject of the invention. The RNA polymerase proteins are in fact extremely conserved according to the species, which makes it possible to find amino acid sequences which align with one another, and thus to choose degenerate oligonucleotides for amplifying the intergenic sequences.

Please amend the paragraph beginning at page 7, line 32 as follows:

The pairs of primers described by the sequences: (a sequence chosen from the sequences SEQ ID [[No.]] NO: 1 to SEQ ID No. 8)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 9 to SEQ ID [[No.]] NO: 11) are used to perform a first amplification of

the intergenic, IGR, of the bacteria. A second, more specific, amplification can then be carried out using pairs of primers which hybridize within the first amplified region, and which are described by the sequences: (a sequence chosen from the sequences SEQ ID [[No.]] NO: 12 to SEQ ID [[No.]] NO: 15)/(a sequence chosen from the sequence SEQ ID No. 16 to SEQ ID [[No.]] NO: 31).

Please amend the paragraph beginning at page 8, line 6, as follows:

SEQ ID [[No.]] NO: 1 GGNGAYAARY TNGCNGGNAG NCAYGG

SEQ ID [[No.]] NO: 2 GGNGAYAARY TNGCNGGNCG NCAYGG

SEQ ID [[No.]] NO: 3 GGNGAYAARY TNGCNAAYAG NCAYGG

SEQ ID [[No.]] NO: 4 GGNGAYAARY TNGCNAAYCG NCAYGG

SEQ ID [[No.]] NO: 5 GGNGAYAARA TGGCNGGNMG NCAYGG

SEQ ID [[No.]] NO: 6 GGNGAYAART TYGCNTCNMG NCAYGG

SEQ ID [[No.]] NO: 7 GGNGAYAART TYGCNAGYMG NCAYGG

SEQ ID [[No.]] NO: 8 GGNGAYAART TYGCNACNMG NCAYGG

SEQ ID [[No.]] NO: 9 AAYGCNGAYT TYGAYGGNGA YCARAT

SEQ ID [[No.]] NO: 10 AAYGCNGAYT TYGAYGGNCA RATGGC

SEQ ID [[No.]] NO: 11 AAYGCNGAYT TYGAYGGNGA YGARAT

SEQ ID [[No.]] NO: 12 GGNGGNCARM GNTTYGGNGA RATGGA

SEQ ID [[No.]] NO: 13 GGNGGNCAYG GNTTYGGNGA RATGGA

SEQ ID [[No.]] NO: 14 GGNGGNCARW SNTTYGGNGA RATGGA

SEQ ID [[No.]] NO: 15 GGNGGNNTNM GNTTYGGNGA RATGGA

SEQ ID [[No.]] NO: 16 GGNAARCGNG TNGAYTAYTC NNGGNMG

SEQ ID [[No.]] NO: 17 GGNAARCGNG TNGAYTAYAG NGGNMG

Please amend the paragraph beginning at page 8, line 24, as follows:

SEQ ID [[No.]] NO: 18 GGNAARAGNG TNGAYTAYTC NGGNMG

SEQ ID [[No.]] NO: 19 GGNAARAGNG TNGAYTAYAG NGGNMG

SEQ ID [[No.]] NO: 20 GGNAARCGNG GNGAYTAYTC NGTNMG

SEQ ID [[No.]] NO: 21 GGNAARCGNG GNGAYTAYAG NGTNMG

SEQ ID [[No.]] NO: 22 GGNAARAGNG GNGAYTAYTC NGTNMG

SEQ ID [[No.]] NO: 23 GGNAARAGNG GNGAYTAYAG NGTNMG

SEQ ID [[No.]] NO: 24 GGNAARCGNG TNGAYTTYTC NGGNMG

SEQ ID [[No.]] NO: 25 GGNAARCGNG TNGAYTTYAG NGGNMG

SEQ ID [[No.]] NO: 26 GGNAARAGNG TNGAYTTYTC NGGNMG

SEQ ID [[No.]] NO: 27 GGNAARAGNG TNGAYTTYAG NGGNMG

SEQ ID [[No.]] NO: 28 GGNAARCGNG TNGAYTTYTC NGCNMG

SEQ ID [[No.]] NO: 29 GGNAARCGNG TNGAYTTYAG NGCNMG

SEQ ID [[No.]] NO: 30 GGNAARAGNG TNGAYTTYTC NGCNMG

SEQ ID [[No.]] NO: 31 GGNAARAGNG TNGAYTTYAG NGCNMG

Please amend the paragraph beginning at page 9, line 5, as follows:

The pairs of primers described by the sequences: (a sequence chosen from the sequence SEQ ID [[No.]] NO: 53 to SEQ ID [[No.]] NO: 54 are used to amplify a the intergenic region, IGR, of the bacteria.

Please amend the paragraph beginning at page 9, line 9, as follows:

FO SEQ ID [[No.]] NO: 53 GGNGGNCANN SNTTYGGNGA RATGGA

RP SEQ ID [[No.]] NO: 54 AAYGCNGAYT TYGAYGGNGA YSARAT

FO SEQ ID [[No.]] NO: 55 GGNGGNCARM GNTTYGGNGA RATGGA  
SEQ ID [[No.]] NO: 56 GGNGGNCAYG GNTTYGGNGA RATGGA  
SEQ ID [[No.]] NO: 57 GGNGGNCARW SNTTYGGNGA RATGGA  
SEQ ID [[No.]] NO: 58 GGNGGNNTNM GNTTYGGNGA RATGGA  
SEQ ID [[No.]] NO: 59 AAYGCNGAYT TYGAYGGNGA YCARAT  
SEQ ID [[No.]] NO: 60 AAYGCNGAYT TYGAYGGNCA RATGGC  
SEQ ID [[No.]] NO: 61 AAYGCNGAYT TYGAYGGNGA YGARAT

Please amend the paragraph beginning at page 9, line 19, as follows:

These primers were designed based on the study of the degeneracy of conserved protein motifs corresponding to rpoB and/or encoded by the rpoB gene:

beta 2 I;

coryneb/bif/actinom/camp/pseudom/salmon/esch/vibrio/clos/bact/hel/citrob  
/prot/haf/yers/past/actinob/aer

SEQ ID [[No.]] NO: 55 GGNGGNCARM GNTTYGGNGA RATGGA (8 deg)

Beta 2 ii: bacillus

SEQ ID [[No.]] NO: 56 GGNGGNCAYG GNTTYGGNGA RATGGA (7 deg)

beta 2 iii: helicobacter mustelae

SEQ ID [[No.]] NO: 57 GGNGGNCARW SNTTYGGNGA RATGGA (8 deg)

beta 2 iv: archae (methano)

SEQ ID [[No.]] NO: 58 GGNGGNNTNM GNTTYGGNGA RATGGA (9 deg)

FO : 2 I/II/III: GGNGGNCANN SNTTYGGNGA RATGGA

(SEQ ID [[No.]] NO: 53)

Please amend the paragraph beginning at page 10, line 1, as follows:

For the reverse sequences, determined based on the degeneracy of conserved protein motifs corresponding to rpoC and/or encoded by the rpoC gene

beta p 2 i:

coryneb/bif/actinom/bac/camp/pseudom/salmon/esch/vibrio/clos/bact/hel/citro  
b/prot/haf/yers/past/actinob/aer/staph/lactob/enteroc/lactoc

SEQ ID [[No.]] NO: 59 AAYGCNGAYT TYGAYGGNGA YCARAT (8 deg)

beta p 2 ii: archae (methano)

SEQ ID [[No.]] NO: 61 AAYGCNGAYT TYGAYGGNGA YGARAT (8 deg)

beta p 2 iii: streptoc

SEQ ID [[No.]] NO: 60 AAYGCNGAYT TYGAYGGNCA RATGGC (7 deg)

RP :P 2 i/ii: AAYGCNGAYT TYGAYGGNGA YSARAT

(SEQ ID [[No.]] NO: 54)

« REVERSE » ATYTSRTCNC CRTCAARTC NGCRTT

(SEQ ID [[No.]] NO: 62)

Please amend the paragraph beginning at page 10, line 19, as follows:

A subject of the invention is also the genomic sequences of microorganisms which may be amplified by the primers according to the invention, in particular the pairs of primers: (a sequence chosen from the sequences SEQ ID [[No.]] NO: 1 to SEQ ID [[No.]] NO: 8)/(a sequence chosen from sequences SEQ ID [[No.]] NO: 9 to SEQ ID [[No.]] NO: 11), and the pairs of primers: (a sequence chosen from the sequences SEQ ID [[No.]] NO: 12 to SEQ ID [[No.]] NO: 15)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 16 to SEQ ID [[No.]] NO: 31). Amplification with pairs of primers: (a sequence chosen from the sequences

SEQ ID [[No.]] NO: 53, SEQ ID [[No.]] NO: 55 to SEQ ID [[No.]] NO: 58)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 54, SEQ ID [[No.]] NO: 59 to SEQ ID [[No.]] NO: 61) is also envisioned.

Please amend the paragraph beginning at page 10, line 30, as follows:

Thus, a subject of the invention is also in particular a sequence from SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138, which correspond to the hypervariable intergenic regions of the rpoB operon or various organisms. A subject of the invention is also a fragment of a minimum of 20 bases, preferably 30 bases, more preferably 50 bases, even more preferably 75 bases, most preferably 100 bases of one of the sequences SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138, or the sequences complementary thereto, it being possible for said fragment to be used to define organism-specific primers, or for the identification of organisms, in particular by hybridization.

Please amend the paragraph beginning at page 11, line 5, as follows:

Thus, the DNA chip according to the invention preferably has, at its surface, a plurality of oligonucleotides (a minimum of two) comprising fragments chosen from the fragments of the sequences SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138 defined above, thus allowing the identification of microorganisms. The length of these oligonucleotides can be determined by those skilled in the art, as a function of the hybridization conditions which they intend to use. Oligonucleotides approximately 50 bases long are thus envisioned.

Please amend the paragraph beginning at page 11, line 31, as follows:

Preferably, the primers described by the sequences SEQ ID [[No.]] NO: 32 and SEQ ID [[No.]] NO: 33 are used to amplify the intergenic region, IGR, of E. coli and of Enterobacteriaceae.

ENT-BDEG:

CTGGAYGTGA ARRTNGGYGA YATYGT (SEQ ID [[No.]] NO: 32)

ENT-ADEG:

ANNACNGTNG CRGTRGTGGT RCCGTC (SEQ ID [[No.]] NO: 33)

Please amend the paragraph beginning at page 12, line 6, as follows:

Other degenerate primers can also be used to implement the protocol according to the invention, in particular any primer chosen from the sequences SEQ ID [[No.]] NO: 34 to SEQ ID [[No.]] NO: 52.

UNI-ADEG 1:

GGNGAYGGNA CNACNACNGC NACNNT (SEQ ID [[No.]] NO: 34)

UNI-ADEG 2:

GGNGAYGGNA CNACNACNTG NTCNNT (SEQ ID [[No.]] NO: 35)

ENT-BNEW:

AANMTTCGTC CNYTRCANGA YCGNGT (SEQ ID [[No.]] NO: 36)

CLO-BNEW2:

ATNARRCCAY TWGGWGAYMG NGTWGT (SEQ ID [[No.]] NO: 37)

BIF-BNEW:

AARCCRCTCG AGGACMRNRT NSTSGT (SEQ ID [[No.]] NO: 38)

UNI-A3:

GGNGAYGGNA CNAANACNGC NACNNT (SEQ ID [[No.]] NO: 39)

BIF-BNEW2:

ATCAAGCCNC TMGRRGACMR SRTNST (SEQ ID [[No.]] NO: 40)

HEL-BNEW:

NTNCANCCNT TNGGNGANAG NGTNTT (SEQ ID [[No.]] NO: 41)

CAM-BNEW:

NTNCANCCNT TNGGNAANCG NGTNCT (SEQ ID [[No.]] NO: 42)

BACT-BNEW:

NTNAANCCNT TNGCNGANCG NGTNCT (SEQ ID [[No.]] NO: 43)

CHLA-BNEW:

NTNAANCCNT TNGGNGANAG NATNTT (SEQ ID [[No.]] NO: 44)

MYCP-BNEW:

NTNAAACCNNTNGGNAANCGNGTNAT (SEQ ID [[No.]] NO: 45)

STA-BNEW:

NTNAAACCNNTNGGNAANCGNGTNAT (SEQ ID [[No.]] NO: 46)

LACC-BNEW:

TTGAAACCNTTAGNGRAYCGYGTRST (SEQ ID [[No.]] NO: 47)

LACB-BNEW:

TTAMARCCAWTMGGNGATCGNGTNRT (SEQ ID [[No.]] NO: 48)

CLO-BNEW3:

ATNANACCANTNGGNGACAGNGTNGT (SEQ ID [[No.]] NO: 49)

ENT-BNEW2:

NTNCGNCCNTTNCANGANCGNGTNAT (SEQ ID [[No.]] NO: 50)

LEG-BNEW:

NTNCGNCCNTTNCANGANCGNGTNGT (SEQ ID [[No.]] NO: 51)

AER-BNEW:



NTNCGNCCNCTNCANGANCGNGTNAT (SEQ ID [[No.]] NO: 52)

LACB-BNEW2:

MARCCNNTNG GNGAYMGNGT NATNGT (SEQ ID [[No.]] NO: 139)

Please amend the paragraph beginning at page 13, line 17, as follows:

These primers are also subjects of the present invention. Preferably, the detection of a microorganism is carried out using a pair of primers SEQ ID [[No.]] NO: 32/ SEQ ID [[No.]] NO: 33, or (SEQ ID [[No.]] NO: 34, SEQ ID [[No.]] NO: 35 or SEQ ID [[No.]] NO: 39)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 36 to SEQ ID [[No.]] NO: 38 or SEQ ID [[No.]] NO: 40 to SEQ ID [[No.]] NO: 52).

Please amend the paragraph beginning at page 13, line 23, as follows:

The sequences SEQ ID [[No.]] NO: 36 to SEQ ID [[No.]] NO: 38 and/or SEQ ID [[No.]] NO: 40 to SEQ ID [[No.]] NO: 52 and/or SEQ ID [[No.]] NO: 139, used in particular in amplification reactions with sequences SEQ ID [[No.]] NO: 34, SEQ ID [[No.]] NO: 35 and/or SEQ ID [[No.]] NO: 39, make it possible, respectively, to detect the microorganisms and species listed below. One or more pair(s) of sequences may be used in an amplification reaction.

Please amend the paragraph beginning at page 13, line 29, as follows:

Thus, the sequences according to the present invention make it possible in particular to detect microorganisms of the following genera and families: Lactococcus (SEQ ID [[No.]] NO: 39), Bifidibacterium (SEQ ID [[No.]] NO: 38 and/or 40), Mycobacterium (SEQ ID [[No.]] NO: 40), Helicobacter (SEQ ID [[No.]] NO: 41), Campylobacter (SEQ ID [[No.]]

NO: 42), Bacteroides (SEQ ID [[No.]] NO: 43), Chlamydia (SEQ ID [[No.]] NO: 44), Mycoplasma (SEQ ID [[No.]] NO: 45), Staphylococcus (SEQ ID [[No.]] NO: 46), Lactococcus and/or Streptococcus (SEQ ID [[No.]] NO: 47), Lactobacillus and/or Bacillus (SEQ ID [[No.]] NO: 48), Clostridium (SEQ ID [[No.]] NO: 37 and/or SEQ ID NO: 49), Enterobacteriaceae (SEQ ID [[No.]] NO: 36 and/or SEQ ID NO: 50), Pasteurella and/or Haemophilus (SEQ ID [[No.]] NO: 50), Neisseria and/or Legionella (SEQ ID [[No.]] NO: 51), Aeromonas and/or Bordetella (SEQ ID [[No.]] NO: 52), Lactobacillus and/or Bacillus (SEQ ID [[No.]] NO: 139).

Please amend the paragraph beginning at page 14, line 7, as follows:

The subject of the invention is also the genomic sequences of microorganisms which can be amplified using the primers according to the invention, in particular the pairs of primers SEQ ID [[No.]] NO: 32/ SEQ ID [[No.]] NO: 33, and (SEQ ID [[No.]] NO: 34, SEQ ID [[No.]] NO: 35 or SEQ ID [[No.]] NO: 39)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 36 to SEQ ID [[No.]] NO: 38, SEQ ID [[No.]] NO: 40 to SEQ ID [[No.]] NO: 52 or SEQ ID [[No.]] NO: 139).

Please amend the paragraph beginning at page 14, line 14, as follows:

Thus, a subject of the invention is also in particular a sequence from SEQ ID [[No.]] NO: 140 to SEQ ID [[No.]] NO: 189, which correspond to the hypervariable intergenic regions of the GroESL operon of various organisms. A subject of the invention is also any fragment of a minimum of 20 bases, preferably 30 bases, more preferably 50 bases, even more preferably 75 bases, most preferably 100 bases of one of the sequences SEQ ID [[No.]] NO: 140 to SEQ ID [[No.]] NO: 189, or the sequences complementary thereto, it being

possible for said fragment to be used to define organism-specific primers, or for the identification of organisms, in particular by hybridization.

Please amend the paragraph beginning at page 14, line 23, as follows:

Thus, the DNA chip according to the invention preferably has, at its surface, a plurality of oligonucleotides (a minimum of two) comprising fragments chosen from the fragments of the sequences SEQ ID [[No.]] NO: 140 to SEQ ID [[No.]] NO: 189 defined above, thus allowing the identification of the microorganisms. The length of these oligonucleotides can be determined by those skilled in the art, as a function of the hybridization conditions, which they intend to use. Oligonucleotides approximately 50 bases long are thus envisioned.

Please amend the paragraph beginning at page 17, line 19, as follows:

Finally, the region of interest of the beta operon was amplified, i.e. the region amplifiable by PCR, using the two corresponding degenerate primers (FO and RP: SEQ ID [[No.]] NO: 53 and SEQ ID [[No.]] NO: 54) for selection of bacteria in order to establish the sequence thereof and to test them by hybridization on a nylon membrane so as to validate this specificity. These sequences were also aligned to their homologs available on GenBank in order to observe this specificity by bioinformatics.

Please amend the paragraph beginning at page 20, line 22, as follows:

Thus, the primers SEQ ID [[No.]] NO: 34 and SEQ ID [[No.]] NO: 35 were defined after alignment of the sequences corresponding to more than 100 species of living organisms (prokaryotes and eukaryotes, not shown).

Please amend the paragraph beginning at page 20, line 26, as follows:

The sequences SEQ ID [[No.]] NO: 36 to SEQ ID [[No.]] NO: 52, and in particular SEQ ID [[No.]] NO: 139, correspond to complementary sequences which can be used to amplify microorganisms of diverse genera and/or families.

Please amend the paragraph beginning at page 20, line 30, as follows:

As regards the primers SEQ ID [[No.]] NO: 32 and SEQ ID [[No.]] NO: 33, they were defined based on the conserved sequences of the GroES and GroEL genes of *E. coli*, using the degenerative genetic code.

Please amend the paragraph beginning at page 21, line 11, as follows:

Analysis of the amplicates makes it possible to show that it is possible to amplify, using the primers SEQ ID [[No.]] NO: 32 and SEQ ID [[No.]] NO: 33, the intergenic region of various enterobacteria, such as *Escherichia coli*, *Enterobacter cloacae*, *Morganella morganii*, *Serratia liquefaciens*, *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Citrobacter freundii* or *Klebsiella oxytoca*. The amplified region varies in length, according to the species, from 400 to 500 base pairs (bp). Use of the pair SEQ ID [[No.]] NO: 34 and SEQ ID [[No.]] NO: 36 gives amplicates of between 550 and 650 bp in length.

Please amend the paragraph beginning at page 21, line 20, as follows:

Use of the pairs: (SEQ ID [[No.]] NO: 34, SEQ ID [[No.]] NO: 35 or SEQ ID [[No.]] NO: 39)/(a sequence chosen from the sequences SEQ ID [[No.]] NO: 36 to SEQ ID [[No.]] NO: 38 or SEQ ID [[No.]] NO: 40 to SEQ ID [[No.]] NO: 52, or SEQ ID [[No.]] NO: 139)

makes it possible to amplify sequences specific to certain families and species, and to identify the organisms of these families or species.

Please amend the paragraph beginning at page 22, line 20, as follows:

Analysis of the amplicates makes it possible to show that it is possible to amplify, using the primers SEQ ID [[No.]] NO: 32 and SEQ ID [[No.]] NO: 33, the intergenic region of various Enterobacteria, such as *Escherichia coli*, *Enterobacter cloacae*, *Morganella morganii*, *Serratia liquefaciens*, *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Citrobacter freundii* or *Klebsiella oxytoca*. The amplified region varies in length, according to species, from 400 to 500 base pairs (bp). Use of the pair SEQ ID [[No.]] NO: 34 and SEQ ID [[No.]] NO: 36 gives amplicates of between 550 and 650 bp in length.

Please amend the paragraph beginning at page 23, line 4, as follows:

Analysis of the amplicates makes it possible to show that it is possible to amplify, using the pair of primers SEQ ID [[No.]] NO: 53 and SEQ ID [[No.]] NO: 54, the intergenic region of the various bacteria, such as *Escherichia coli*, *Clostridium leptum*, *Klebsellia oxytoca*, *Lactococcus lactis*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus mirabilis*, *Serratia liquefaciens*, *Morganella morganii*, *Enterobacter cloacae* or *Ruminococcus hydrogenotrophicus*.

Please amend the paragraph beginning at page 23, line 1, as follows:

Figures 4 to 6 show a specificity detection as a function of the organisms, although some crosshybridization reactions may exist. These reactions may be reduced by choosing probes which are shorter and located among the hypervariable intergenic sequences, as

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defined by SEQ ID [[No.]] NO: 63 to SEQ ID [[No.]] NO: 138 (rpoN) or SEQ ID [[No.]]  
NO: 40 to SEQ ID [[No.]] NO: 189 (GroESL).